



**Figure S3. Testing the ability of electron transport chain inhibitors and a proton ionophore to increase *S. aureus* biofilm sensitivity to chloroxylenol.** (A to D) Biofilm disruption assays on plastic were performed with *S. aureus* (Sa) Newman, chloroxylenol (Chlor) at 100 µg/ml, and the specified concentrations of 3-nitropropionic acid (3-NP) (A), Antimycin A (B), Sodium azide

(azide) (C), or oligomycin (Oligo) (D). Biofilms were grown for 6 hours, exposed to the above treatments for 18 hours, and *S. aureus* biofilm CFU were determined. (E) Biofilm disruption assays on plastic were performed with *S. aureus* (Sa) JE2 parental strain or the specified transposon mutant, and chloroxymenol (Chlor) at 100 µg/ml. Each column displays the average from at least two biological replicates, each with three technical replicates. Error bars indicate SD. ns, not significant; \*,  $P < 0.05$ , \*\*\*,  $P < 0.001$ , by ordinary one-way ANOVA and Tukey's multiple comparison post-test. (F) Membrane potential was measured using the fluorescent dye DiOC<sub>2</sub> following exposure to carbonyl cyanide 3-chlorophenylhydrazone (CCCP) at 5 µM, HQNO at 100 µg/ml, Antimycin A at 100 µg/ml, or *P. aeruginosa* PA14 wild-type or  $\Delta pqsLpvdApchE$  deletion mutant supernatant for 24 h. DiOC<sub>2</sub> fluorescence was measured at 680 nm following excitation at 485 nm. Results are reported as Fluorescence / OD<sub>600</sub>. Each column displays the average from two biological replicates, each with three technical replicates. (G) Biofilm disruption assays on plastic were performed with *S. aureus* (Sa) Newman, chloroxymenol (Chlor) at 100 µg/ml, and CCCP at 5 µM or 25 µM. Biofilms were grown for 6 hours, exposed to the above treatments for 18 hours, and *S. aureus* biofilm CFU were determined. Each column displays the average from at least two biological replicates, each with three technical replicates. Error bars indicate SD. ns, not significant; \*,  $P < 0.05$ , by ordinary one-way ANOVA and Tukey's multiple comparison post-test.